4500
FS-SO-2203-1.37
(Problem 2 RWUD-1978)

September 1978
Study Plan Amendment Summary
J. Robert Bridges - Alexandria, La.

NITROGEN UTILIZATION BY THE SOUTHERN PINE BEETLE (SPB)

The original study plan proposed to determine the efficiency of conversion of digested nitrogen (N) to body substance (E.C.D.(N)). To estimate E.C.D.(N) the nitrogenous excretory products of SPB larvae must be determined. Because these excretory products are difficult to quantitate and may be utilized or metabolized by symbiotic microorganisms, reliable estimates of E.C.D.(N) could not be obtained. Therefore, in the amended study plan, the efficiency of conversion of ingested nitrogen to body substance E.C.I.(N) will replace E.C.D.(N). The E.C.I.(N) is easier to estimate and should provide an overall measure of N utilization because it takes into account the digestibility of the food as well as the conversion of digested food to body substance.

UNITED STATES DEPARTMENT OF AGRICULTURE

FOREST SERVICE

4500
FS-SO-2203-1.37
(Problem 2 RWUD 1978)

STUDY PLAN AMENDMENT

NITROGEN UTILIZATION BY THE SOUTHERN PINE BEETLE (SPB)

J. Robert Bridges

RWU 2203

Southern Forest Experiment Station

U.S. GOVERNMENT PRINTING OFFICE : 1973 O—522-184

6200—M7 (10/73)

FS-SO-2203-1.37

STUDY PLAN AMENDMENT

NITROGEN UTILIZATION BY THE SOUTHERN PINE BEETLE (SPB)

Prepared by:

J. Robert Bridges — 9/19/78
J. Robert Bridges — Date
Research Entomologist
RWU-2203

Approved by:

Peter L. Lorio, Jr. — 9/20/78
Peter L. Lorio, Jr. — Date
Project Leader
RWU-2203

NITROGEN UTILIZATION BY THE SOUTHERN PINE BEETLE (SPB)

STUDY PLAN AMENDMENT

The original Study Plan proposed to determine the effeciency of conversion of digested nitrogen (N) to body substance (E.C.D.(N). The E.C.D.(N) was to be used to predict whether the increased nitrogen content of pine inner bark due to the symbiotic microorganisms is beneficial to the beetle.

It is now planned to replace the E.C.D.(N) with another nutritional index, the E.C.I.(N). This index measures the effeciency of conversion of ingested nitrogen to body substance. It can be calculated from the following:

$$E.C.I.(N) = \frac{\text{amount of N in body}}{\text{amount of N in body + amount of N in frass}}$$

The E.C.I.(N) will give an overall measure of nitrogen utilization and will vary with both the digestibility of a food and the proportional amounts of the digestible portions of that food which are converted to body substance. On the other hand, the E.C.D.(N) does not take into account changes in the digestibility of the food. Thus, the E.C.I.(N) should give a better overall measure of N utilization than E.C.D.(N).

The E.C.I.(N) is also much easier to estimate. It can be calculated from measurements of the total N in SPB larvae and frass, which are both relatively easy to determine. To obtain an estimate

of the E.C.D.(N) the nitrogenous excretory products must be determined. Preliminary experiments showed that the excretory products uric acid, allantoic acid, and allantoin were all found in SPB larval frass. However, the procedures for quantitating these compounds are difficult and time consuming. It is also possible, perhaps likely, that symbiotic microorganisms in the frass may metabolize the nitrogenous excretory products and prevent their accurate quantitation. Therefore, without a better understanding of the possible role of symbiotic microorganisms in the metabolism of nitrogen excretory products, the E.C.D.(N) cannot be measured with confidence.

It is for these reasons that the E.C.I.(N) will be used instead of E.C.D(N). Other aspects of the study such as total nitrogen determination, collection of larvae and frass, and statistical analysis of the data will remain as originally planned.

UNITED STATES DEPARTMENT OF AGRICULTURE
FOREST SERVICE

SO

REPLY TO: 1630 Written Information May 21, 1976

SUBJECT: Study Plan FS-SO-2203-1.37 (~~ESPBP~~)
"Nitrogen Utilization by the Southern Pine Beetle (SPB)"

TO: T. A. Harrington, AD-WG

Attached is study plan FS-SO-2203-1.37 entitled "Nitrogen Utilization by the Southern Pine Beetle (SPB)". This study plan is under the Expanded SPB Program.

J. ROBERT BRIDGES
Research Chemist
Forest Insect Research

Enclosure

JRBridges:bfs

4500
FS-SO-2203-1.37 (ESPBP)
(Problem 2)

Study Plan Summary
J. Robert Bridges - Alexandria, La.

NITROGEN UTILIZATION BY THE SOUTHERN PINE BEETLE (SPB)

This study will investigate the relationship of the Southern Pine Beetle and its symbionts from a nutritional standpoint. The study will determine the efficiency of conversion of digested nitrogen into body substance in order to ascertain whether nitrogen is the limiting nutrient for growth of the SPB and thus whether the increased nitrogen content of pine inner bark due to the symbiotic microorganisms is beneficial to the beetle.

UNITED STATES DEPARTMENT OF AGRICULTURE

FOREST SERVICE

4500
FS-SO-2203-1.37 (ESPBP)
(Problem 2)

STUDY PLAN

NITROGEN UTILIZATION BY THE SOUTHERN PINE BEETLE (SPB)

J. Robert Bridges
Research Entomologist

RWU 2203

Forest Insect Research Project
Alexandria, Louisiana 71301

Southern Forest Experiment Station

U.S. GOVERNMENT PRINTING OFFICE : 1973 O—522-184

6200—M7 (10/73)

4500
FS-SO-2203-1.37 (ESPBP)
(Problem 2)

STUDY PLAN

NITROGEN UTILIZATION BY THE SOUTHERN PINE BEETLE (SPB)

Prepared by:

J. Robert Bridges 4/14/76
J. Robert Bridges (Date)
Research Chemist
RWU 2203

Approved by:

Stanley J. Barras 4/14/76
Dr. Stanley J. Barras (Date)
Project Leader - RWU 2203

4500
FS-SO-2203-1.37
(Problem 2)

STUDY PLAN

NITROGEN UTILIZATION BY THE SOUTHERN PINE BEETLE (SPB)

By

J. Robert Bridges

The SPB lives in symbiotic relationship with many microorganisms including 2 species of mycangial fungi (Barras and Perry, 1972). The association between the SPB and its symbiotic fungi is obligatory because growth and development of the beetle are much reduced in the absence of these fungi (Barras, 1973). However, the reasons for this dependency of the SPB on its symbionts are not known.

Because the total nitrogen content of pine bark is relatively low (Hodges et al., 1968), this nutrient might limit the growth of the SPB, and the symbiotic microorganisms could function to provide the necessary additional nitrogen to the beetle. It is known that colonization of pine inner bark tissue by the symbiotic microbial complex does increase the concentration of total nitrogen in the bark (Hodges et al., 1968) but whether this increase in total nitrogen benefits the beetle is not known. This is one of a series of studies to analyze the symbiotic association from a nutritional standpoint to determine whether the increase in nitrogen

content of inner bark by the microbial complex is beneficial to the beetle and, therefore, whether nitrogen nutrition is the basis for the symbiotic relationship.

The major goal of the study is to estimate the efficiency of conversion of digested nitrogen (N) to body substance, (E.C.D.[N]). This nutritional index can be determined from the following formula (Waldbauer, 1968):

$$E.C.D.(N) = \frac{\text{amount of N in body}}{\text{amount of N in body + amount of N in excretory products.}}$$

Animals developing on a limiting nitrogen source would be expected to have relatively high E.C.D.(N)'s because most of the N is retained in the body rather than being excreted. Animals growing on substrates with excess nitrogen would be expected to excrete the excess thus decreasing the E.C.D.(N).

The calculation of the E.C.D.(N) for SPB larvae should allow one to predict whether there is enough nitrogen in uninfested bark to support growth and development. Hodges et al. (1968) reported that the uninfested inner bark of loblolly pine contained 24% less total nitrogen than inner bark containing the symbiotic microorganisms. In order for the SPB larvae to obtain enough nitrogen for growth and development from a given amount of uninfested bark, they must utilize the available nitrogen 24% more efficiently. If from this study, the E.C.D.(N) of larvae feeding on bark containing symbiotic microorganisms is found to be above 76%, then it can be predicted that the poor growth and development of SPB on unifested phloem (Barras, 1973) is due in part to a nitrogen shortage since larvae could not increase their efficiency abouve 100%.

METHODS

To calculate the E.C.D.(N) the following measurements will be needed:

a) Total nitrogen content of SPB larvae

b) Amount of frass produced/larva

c) Amount of nitrogenous excretory products (uric acid) in frass.

Analyses will also be made to determine the total nitrogen content of inner bark colonized by SPB symbiotic complex and of uninfested control bark.

To obtain these measurements, a healthy loblolly pine tree will be felled and the lower part cut into 6 bolts each approximately 50 cm in length. Each bolt will be inoculated with 16 pairs of adult SPB. One portion of the bolt will be left uninoculated in order to obtained uninfested bark (control) bark tissue. The bolts will be stored in the laboratory for 2-3 weeks to allow for the development of mature inner bark larvae and for development of the SPB symbiotic microbial complex. Bark tissue will be obtained for total N analysis from each of the 6 bolts from areas adjacent to the larval galleries (within 3-5 mm). Control bark tissue will be obtained from a portion of each bolt that does not contain beetles or microorganisms. Three of these bolts also will be dissected to obtain larvae and frass. Larvae, frass, and bark tissue from a single bolt will be pooled, lyophilized, and weighed.

Aliquots of larvae and bark tissue will be analyzed for total nitrogen by the method of Hodges et al. (1968). Approximately 10 mg of tissue will be required for each analysis.

Frass will be analyzed for uric acid, allantoin, allantoic acid, and urea using the paper chromatographic methods of Fink et al. (1963). Quantitative determination of the uric acid content of frass will be done by the method of Kageyama (1971).

STATISTICAL ANALYSES OF DATA

Values for the E.C.D.(N) obtained for each of the 3 replications (bolts) will be averaged to obtain the mean and standard deviation.

Data for the total nitrogen content of infested versus uninfested bark tissue will be analyzed using an analysis of variance as summarized below:

Source of variation	df
Bolts (replications)	5
Type of bark	1
Error	5
Total	11

PERSONNEL ASSIGNMENT

J. Robert Bridges	Research Chemist
Faye Guinn	Biological Lab Technician

LITERATURE CITED

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FS-SO-2203-1.37

STUDY PLAN AMENDMENT

NITROGEN UTILIZATION BY THE SOUTHERN PINE BEETLE (SPB)

Prepared by:

J. Robert Bridges — 9/19/78
J. Robert Bridges — Date
Research Entomologist
RWU-2203

Approved by:

Peter L. Lorio, Jr. — 9/20/78
Peter L. Lorio, Jr. — Date
Project Leader
RWU-2203

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SO-4000-5 (REV. 12/71)

BIOMETRICS REVIEW ROUTING

ROUTING

~~1) Biometrics — see remarks~~
~~2) RWU 2203~~
~~3) ADxWG~~
3) ~~Biometrics~~
4) ~~RWU 2203~~
5) AD-WG

RESEARCH WORK UNIT

☒ STUDY PLAN ☐ MANUSCRIPT ☐ RESPONSE TO BIOMETRICS REVIEW ☐ _____ (OTHER)

FS-SO-2203-1.37 (STUDY NUMBER) Nitrogen Utilization by the Southern Pine Beetle (SPB) (TITLE)

J. Robert Bridges (AUTHOR SIGNATURE) 3-12-76 (DATE)

J. Robert Bridges, Research Chemist

REMARKS:

Main purpose is to determine the E.C.D.(N) with some chromatography work on other compounds. Probably needs little biometrics review.

SUBMITTED: Stanley J. Barras (PROJECT LEADER SIGNATURE) RWU 2203 3-12-76 (DATE)

AD

☐ FORWARDED FOR BIOMETRICS REVIEW ☐ BIOMETRICS REVIEW WAIVED

(ASSISTANT DIRECTOR SIGNATURE) (DATE)

BIOMETRICS SERVICES

☐ NO COMMENTS ☐ COMMENTS BELOW ☐ COMMENTS ~~ATTACHED~~
☒ COMMENTS MADE BY PHONE WRITTEN ON REVIEW COPY

IN 3/22/76

T. R. Dell (BIOMETRICIAN SIGNATURE) 3/23/76 (DATE)

OUT 3/23/76

COMMENTS:

Some statistical analysis regarding the reliability of the estimate of average ECD(N) and also for the difference in total nitrogen per unit of weight for the two classes of bark should be considered.

INSTRUCTIONS

1. Project Leader must submit this form with each study plan. Also use this form when manuscripts are submitted for review.
2. Insert desired "Routing" in space at top of form.
3. Be sure to insert proper identification in the "Research Work Unit" block.
4. Completed copies of this form will be filed in the study file in the Assistant Director's and Project Leader's files.

ORIGINAL – RESEARCH WORK UNIT FILE

4500
FS-SO-2203-1.37 (ESPBP)
(Problem 2)

STUDY PLAN

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METHODS

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Aliquots of larvae and bark tissue will be analyzed for total nitrogen by the method of Hodges *et al.* (1968). Approximately 10 mg of tissue will be required for each analysis. Analyses from each bolt will be averaged to obtain the mean total nitrogen concentrations.

Frass will be analyzed for uric acid, allantoin, allantoic acid, and

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PERSONNEL ASSIGNMENT

J. Robert Bridges	Research Chemist
Faye Guinn	Biological Lab Technician

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1st time to Biom

4500
FS-SO-2203-1.37 (ESPBP)
(Problem 2)

STUDY PLAN

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By

J. Robert Bridges

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Data for the total nitrogen content of infested versus uninfested bark tissue will be analyzed using an analysis of variance as summarized below:

Source of variation	df
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Type of bark	1
Error	5
Total	11

PERSONNEL ASSIGNMENT

J. Robert Bridges	Research Chemist
Faye Guinn	Biological Lab Technician

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UNITED STATES DEPARTMENT OF AGRICULTURE
FOREST SERVICE

SO-AL

REPLY TO: 4050 Research Programs

September 25, 1981
(RWU 2203)

SUBJECT: Final Report, FS-SO-2203-1.37, "Nitrogen utilization by the southern pine beetle (SPB)" by J. Robert Bridges

TO: S. J. Barras, AD-W

Enclosed is a copy of subject final report and final report summary for your information.

Bobbie J. Mallard
for PETER L. LORIO, JR.
Project Leader
Forest Insect Research

Enclosure

PLLorio,Jr./bjm